STUDIES ON THE SAPROPHYTISM OF CERTAIN FUNGI CAUSING FOOT-ROTS OF WHEAT

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A THESIS

submitted to the University of Alberta
in partial fulfilment of the requirements for
the degree of

MASTER OF SCIENCE

Edmonton, Alberta
April, 1932

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INTRODUCTION

Foot-rots of wheat are of world-wide importance. They have been reported from all the large wheat growing areas of the world. The prevalence of these diseases in western Canada had not been fully realized until the last few years, but recent work has clearly demonstrated that they are very widespread, and responsible in numerous instances for serious reduction in yields. In this paper the term "foot-rot" is used to designate a rotting of both the basal parts of the stem and the roots, since usually both parts are affected.

There are a number of organisms which cause foot-rots of wheat, some of which have not as yet been recorded in this province. The ones with which we are chiefly concerned in these studies are: Helminthosporium sativum P.K.B., Fusarium graminearum Schawbe, and Ophiobolus graminis Sacc. There are two others of lesser importance which occur in this province, namely, Leptosphaeria herpotrichoides de Not., and Wojnowicia graminis (McAlp.) Sacc.

Both Helminthosporium sativum and Fusarium graminearum have a very wide host range, attacking wheat, barley, rye and numerous species of cultivated and wild grasses. They attack winter as well as spring sown grains, but with us are of greater importance on the latter, especially on spring wheat. They occur in all parts of western Canada. These fungi appear to be able to attack susceptible cereal plants at any stage from the germinating seed to the almost mature plant. When the seedlings are infected some of them die before or soon after emergence as shown in Fig. 1, but those attacked less heavily or later in the season may survive to maturity. They frequently show lesions on the base of the stem, and a rotting of the roots. Infected plants are often stunted, and produce badly shrunken kernels. Oats are almost immune from Helminthosporium sativum, but are quite susceptible to Fusarium graminearum and other related species of Fusarium.

Ophiobolus graminis has a very similar host range to the above two fungi. It attacks both spring and winter wheat causing a disease known as "Take-all", but in the United States winter wheat is attacked almost exclusively. "Take-all" was first reported on spring wheat in the United States during the past year (1931); however, it has been known to occur on spring wheat in Australia for many years, and it also occurs commonly on spring wheat in certain parts of western Canada. Most strains of this

organism are unable to attack oats, though certain European strains are apparently capable of infecting this crop.

Ophiobolus graminis has been reported from widely scattered points in the provinces of Manitoba and Saskatchewan, and in Alberta it has been isolated from wheat grown as far north as Athabasca and as far south as Cardston.

Fig. 1. Seedling blight of wheat on artificially infested soil.

1. Check.
2. Caused by Helminthosporium sativum.
3. Caused by Fusarium graminearum.
4. Caused by Ophiobolus graminis.

All of the fungi under investigation, ~~while~~ though parasitic on cereals and other grasses, are able to live for considerable periods in the absence of any susceptible

hosts. They live as saprophytes on plant residues and apparently also on the humus in the soil. This stage of their life history may be termed saprogenesis as opposed to the parasitic stage known as pathogenesis. A knowledge of the saprophytic existence of these organisms in the soil is essential in order to provide a basis upon which to devise control measures. The aim of crop rotation in disease control is to reduce the amount of inoculum to a minimum, and to provide as unfavourable conditions as possible for the development of the pathogenes. This may be brought about by the growing of resistant crops, and by the use of summer fallow, coupled with cultural practices which tend to make conditions more favourable for the decomposition of plant residues in the soil. Since by the use of immune crops, and by summerfallowing, any pathogenes present are forced to exist as saprophytes if they exist at all, it is of particular interest to know the effect of this saprophytic life on these fungi, for instance to know how long they may survive in this state. The extension of our knowledge in this direction has been the primary object of this investigation.

EXPERIMENTAL METHODS

Method of Isolation from the Soil

The most common method of isolating fungi from the soil is the direct one, by the use of dilutions and poured plates. However, this method is not suitable for comparing the relative numbers of pathogenic fungi in different soils, as indicated by the results of Henry (12), working with Helminthosporium sativum. Another method has therefore been resorted to in these studies, namely, an indirect one, which we have called the "seedling method". This consists in planting sterile seedlings in the different soils, and isolating the pathogenes which attack them. This method was based on the work of Henry (10), who used it to obtain cultures of pathogenic fungi from the soil. However, certain alterations were made in the procedure. Instead of treating the wheat seeds with mercuric chloride and silver nitrate for the production of sterile seedlings, they were treated with hot water as for the control of loose smut. Then the seeds were surface sterilized by dipping them in 70 percent ethyl alcohol, then soaking them in mercuric chloride (1-1000) for two minutes, and finally washing them in 70 percent ethyl alcohol. The seeds were then germinated on potato dextrose agar, and the seedlings allowed to develop until the plumule and roots were about one-half inch long. The sterile seedlings were then

transferred under aseptic conditions to test-tubes containing approximately 10 gm. of the soil to be tested. They were then allowed to grow for 10-12 days at which time they were removed, and the diseased portions of the roots and stems were plated on agar.

This method of seedling purification was probably first used by Bolley (1) in 1913, but he made no statement regarding his methods other than the growing of the seedlings on the agar in test tubes. Since that time a number of other workers have used this method in order to obtain sterile seedlings for inoculation studies.

Wilson (30) in 1915 described a method for seed sterilization, by the use of calcium hypochlorite, which he considered to give very good results. Wilson's method was tried, but did not give very satisfactory results. Hence the method previously outlined was used throughout.

Methods of Isolation from Plant Tissue

In the course of this study it was found to be extremely difficult, and in many cases impossible, to isolate Ophiobolus graminis from plant tissues which were known to be heavily infested with this organism.

The most common method of surface sterilization of plant tissue now being used is the mercuric chloride (1-1000) method. Other workers have found that mercuric chloride is unsuitable to use in the isolation of certain

organisms. Simmonds (24) for example was unable to isolate the organism causing the "browning" root-rot of wheat when he used mercuric chloride to surface sterilize the diseased wheat roots.

The mercuric chloride method has been found quite satisfactory for the isolation of Helminthosporium sativum and Fusarium graminearum, but in the case of Ophiobolus graminis it was found that if any other organisms were present they grew to the exclusion of Ophiobolus, which is a comparatively slow growing fungus.

It was thus necessary to obtain if possible a sterilizing agent which would favour the growth of Ophiobolus, so an experiment was outlined which included the use of a number of such agents, and combinations of these in various concentrations, and for varying lengths of time. These were tested on some artificially inoculated material grown in the greenhouse. The following table gives the substances used and the results obtained.

TABLE 1

Relative value of various chemicals for the surface sterilization of plant tissues for the isolation of Ophiobolus graminis.

No.	Surface sterilizing agent	Conc.	Time	Additional treatment	Successful isolations
1	$HgCl_2$	1-1000	2 min.	Washed in 70% alcohol	1 out of 12
2	"	1-2000	"	" " " "	0 out of 12
3	"	1-1000	"	" " sterile water	0 out of 12
4	$AgNO_3$*	1-gr.-100 cc.	"	Washed in sterile NaCl and Sterile water	4 out of 12
5	"	"	5 min.	Washed in sterile NaCl and sterile water	3 out of 12
6	Calcium hypochlorite	1 gr.-14 cc.	2 min.	Washed in sterile water	0 out of 12
7	"	"	5 min.	Washed in sterile water	0 out of 12
8	H_2O_2	3%	2 min.	Washed in sterile water	0 out of 12
9	"	"	5 min.	Washed in sterile water	0 out of 12
10	Washing in sterile water (Simmond's method)				0 out of 12

* In the silver nitrate method pieces of the infected stems were immersed in a solution of silver nitrate (1-100) for two minutes, then washed in a sterile saturated solution of sodium chloride, and then washed in sterile water after which the pieces were plated on potato dextrose agar.

From the above results it is evident that the percentage of successful isolations of this organism is very low. However, from this preliminary experiment it would appear that the silver nitrate method would give better results than the mercuric chloride method had given in the past.

A larger experiment in the nature of a test comparing the standard mercuric chloride method and the silver nitrate method was then conducted. Ten samples of foot-rot, collected during the disease surveys of 1930 and 1931 and presumably infested with Ophiobolus graminis, were used. Twenty-four pieces of diseased stems were cut from each sample, twelve of which were treated by the mercuric chloride method and twelve by the silver nitrate method. Table 2 gives the results obtained.

TABLE 2

Comparison of mercuric chloride and silver nitrate as surface sterilizing agents of plant tissue for the isolation of Ophiobolus graminis.

Specimen No.	Successful isolations		Specimen No.	Successful isolations	
	$HgCl_2$	$AgNO_3$		$HgCl_2$	$AgNO_3$
247	0 out of 12	2 out of 12	23	0 out of 12	1 out of 12
73	0 out of 12	4 out of 12	201	1 out of 12	6 out of 12
191	0 out of 12	5 out of 12	292	1 out of 12	8 out of 12
2	1 out of 12	1 out of 12	126	0 out of 12	1 out of 12
88	0 out of 12	0 out of 12	141	0 out of 12	0 out of 12

A summary of the above results shows that by the mercuric chloride method, Ophiobolus graminis was isolated from only 3 out of the 10 samples and only 3 isolations out of a possible 120 were made. On the other hand by the silver nitrate method the fungus was isolated from 8 out of the 10 samples and a total of 28 successful isolations were obtained out of a possible 120.

A third experiment along this line was conducted using 25 samples obtained from various parts of the province. Six pieces were cut from each sample and three treated by one method and three by the other. Results are given in Table 3.

TABLE 3

Further comparison of $HgCl_2$ and $AgNO_3$ as a surface sterilizing agent of plant tissue for the isolation of Ophiobolus graminis.

Specimen No.	Successful isolations		Specimen No.	Successful isolations	
	$HgCl_2$	$AgNO_3$		$HgCl_2$	$AgNO_3$
20	0 out of 3	0 out of 3	288	0 out of 3	0 out of 3
83	0 " " "	0 " " "	290	0 " " "	1 " " "
112	0 " " "	0 " " "	294	0 " " "	0 " " "
169	0 " " "	0 " " "	298	0 " " "	0 " " "
178	0 " " "	0 " " "	311	0 " " "	3 " " "
211	0 " " "	0 " " "	342	0 " " "	0 " " "
218	0 " " "	0 " " "	349	0 " " "	0 " " "
232	0 " " "	0 " " "	358	0 " " "	0 " " "
217	0 " " "	1 " " "	379	0 " " "	0 " " "
244	0 " " "	0 " " "	381	0 " " "	0 " " "
260	0 " " "	0 " " "	389	0 " " "	0 " " "
278	1 " " "	1 " " "	395	0 " " "	1 " " "

Summarizing Table 3 we find that the number of successful isolations of Ophiobolus graminis by the silver nitrate method exceeds that by the mercuric chloride method in the ratio of 7:1. The material used in this study was similar to that used in the previous experiment, Table 2, but it will be noted there was a large decrease in the number of successful isolations. This may be due to the fact that the material had been stored for four months in the laboratory.

The results of the three comparative tests of the efficiency of silver nitrate and mercuric chloride as surface sterilizing agents of plant tissue for the isolation of Ophiobolus graminis are summarized in Table 4.

TABLE 4

A summary of the results obtained in the comparison of mercuric chloride and silver nitrate as a surface sterilizing agent of plant tissue for the isolation of Ophiobolus graminis.

Exp. No.	Successful isolations in percent			
	Silver nitrate method		Mercuric chloride method	
1	29.3%	(7 out of 24)	2.8%	(1 out of 36)
2	23.3%	(28 " " 120)	2.5%	(3 " " 120)
3	9.3%	(7 " " 75)	1.3%	(1 " " 75)
Summary	19.2%	(42 " " 219)	2.2%	(5 " " 231)

The above figures show a large increase in favour of the silver nitrate method, and from the constancy of the results of the experiments, and of individual samples, it seems quite safe to recommend silver nitrate as a surface sterilizing agent for the isolation of Ophiobolus graminis from diseased stems.

Inoculation Studies

Certain of the foot-rotting fungi grow readily and multiply in the soil whereas others do not. It has been found that Helminthosporium sativum and Fusarium graminearum are readily increased on sterilized soil when required for use in inoculating seedlings. In some experiments on the pathogenicity of Ophiobolus graminis, sterilized soil was used as a medium for growing the fungus for use as inoculum with rather unsatisfactory results. Tests were therefore made in order to find a more satisfactory medium on which to produce inoculum, and one which would have no detrimental effect on the seedlings. The following media were compared: soil alone, soil to which ten percent corn meal had been added, and a mixture of boiled barley and oats as described by Davis (5). Checks consisting of the uninoculated medium were included in order to determine if the medium had any detrimental effect on the growth of the seedlings. The results are clearly illustrated in Fig. 2.

Fig. 2. Infection of wheat seedlings by Ophiobolus graminis using various types of media as inoculum.

1. Check - uninoculated soil plus ten percent corn meal.
2. Fungus grown on sterilized soil.
3. Fungus grown on sterilized soil plus ten percent corn meal.
4. Fungus grown on a sterilized mixture of boiled barley and oats.

The above results show that the sterilized soil to which had been added ten percent corn meal is a satisfactory medium for the growth of Ophiobolus graminis and for use as inoculum for the infection of wheat seedlings. As great an infection was obtained with this medium as was obtained with the barley oat mixture, and since it contains much less organic matter, and is more easily prepared, it has been used in preference to other media wherever possible.

PERSISTENCE OF FOOT-ROTTING FUNGI

Persistence in the soil

Since it is known that the fungi under discussion can live a saprophytic existence, it is obviously of both scientific interest and much practical importance, to know how long they may survive in this state. A number of factors might be expected to influence their ability to survive, e.g., the substratum on which they are growing, the environmental conditions and the form in which they occur.

Certain of these organisms have been reported as surviving for long periods in diseased seed and stubble, but reports as to their ability to live over in the soil are very meagre. McKinney (17) reports that from field observations it would appear that Ophiobolus graminis cannot exist very long as a saprophyte. Kirby (14) found that screened soil from a "Take-all" area, after 8 months storage in the laboratory would no longer act as inoculum.

An experiment was outlined to determine the effect which various rotations may have on the persistence of these organisms.

In the spring of 1929, seventy-two butter boxes were filled with sterilized soil, and one litre of inoculum, consisting of the various organisms growing on ground oat

hull media, was added at a depth of four inches. The boxes were then placed in the soil to a depth such that the soil in the boxes was level with that outside.

The following organisms were used:

1. Fusarium graminearum
2. Helminthosporium sativum
3. Ophiobolus graminis
4. Leptosphaeria herpotrichoides
5. Mixed culture of the 4 organisms
6. Check

Six rotations were employed including:

1. Bare fallow continuously
2. Wheat continuously
3. Oats continuously
4. Wheat and fallow
5. Wheat and oats
6. Wheat with sweet clover and sweet clover

This gave two replications of each organism in each rotation.

Crops were grown as outlined for 1929, '30 and '31, when isolations were made in three different ways. In the case of the continuous wheat, isolations were made directly from the stubble. The following table gives the results obtained.

TABLE 5.

Isolations from the stubble of the continuous wheat plots inoculated with foot-rotting pathogenes.

Box No.	Soil inoculated with	No. of Colonies of different Organisms isolated
7	Fusarium graminearum	11 Helminthosporium sativum
43		1 Fusarium graminearum
8	Helminthosporium sativum	2 Helminthosporium sativum
44		6 " "
9	Leptosphaeria herpotrichoides	5 Helminthosporium sativum
45		8 " " and 1 Fusarium graminearum
10	Ophiobolus graminis	6 Helminthosporium sativum
46		4 " "
11	Mixed culture	3 Helminthosporium sativum & 5 Fusarium graminearum
47		3 Helminthosporium sativum
12	Check	6 Helminthosporium sativum
48		0

The above results show that serious contamination had taken place, not only with common saprophytes, but also with other parasitic organisms, e.g., the check yielded as many isolations of Helminthosporium sativum as did the boxes inoculated with that particular organism, showing that this method of isolation gives no indication of the persistence of the pathogene.

In the second method of isolation, pieces of the old inoculum were obtained from the boxes by means of a sterile cork borer. Four pieces of this inoculum from the two replications were plated on agar. The results obtained are given in Table 6.

TABLE 6.

Isolations from the old inoculum from the various rotations inoculated with foot-rotting fungi.

Rotation	Organism									
	Fusarium graminearum		Helmintho-sporium sativum		Lepto-sphaeria herpotri-choides		Ophiobolus graminis		Mixed culture	
	Box No.									
Bare fallow	1	+*	2	-	3	-	4	-	5	-
(Cont.)	37	+	38	-	39	-	40	-	41	+ (Fus.
Wheat	7	+	8	-	9	-	10	-	11	-
(Cont.)	43	-	44	+	45	-	46	-	47	-
Oats	13	+	14	-	15	-	16	+	17	-
(Cont.)	49	+	50	-	51	-	52	+	53	-
Wheat & Fallow	19	+	20	-	21	-	22	+	23	-
(Wheat 1931)	55	+	56	-	57	-	58	-	59	-
Wheat & oats	25	+	26	-	27	-	28	-	29	+ (Fus.
(Wheat 1931)	61	-	62	-	63	-	64	-	65	-
Wheat & sweet clover & sweet clover	31	-	32	-	33	-	34	+	35	-
(Wheat & sweet clover 1931)	67	+	68	-	69	-	70	-	71	+ (Fus

* + indicates successful isolations were made.
\- indicates inability to isolate the fungus.

From the above results it appears very evident that the Fusarium graminearum is still persisting under all rotations. However, the absence of the other fungi is not sufficient evidence to even suggest that they do not exist, because the saprophytic organisms which contaminate the culture may grow so quickly as to hide any traces of these less rapidly growing fungi.

The third method of isolation used was the indirect one, by the use of sterile seedlings, which method has previously been described under "methods". Soil samples were taken from the boxes, by the use of a sterile cork borer, and placed in sterile test-tubes under as aseptic conditions as possible. These were then seeded with sterile seedlings, and after two weeks growth diseased portions of the seedlings were plated on agar. The results are summarized in Table 7.

Tests were made only on soils infested with Helminthosporium sativum and Fusarium graminearum, because previous tests had shown it very difficult to isolate Leptosphaeria and Ophiobolus by this method.

TABLE 7.

Isolations from the soil inoculated with Helminthosporium sativum and Fusarium graminearum and subjected to different systems of crop rotation.

Rotation	Fusarium graminearum		Helminthosporium sativum		Check	
	Box No.					
Bare fallow	1	+*	2	+	6	Fus.
	37	-	38	+	42	-
Wheat (cont.)	7	+	8	-	12	H.sat.
	43	-	44	+	48	-
Oats (cont.)	13	+	14	-	18	-
	49	+	50	-	54	-
Wheat & fallow	19	+	20	-	24	-
(Wheat 1931)	55	+	56	+	60	Fus.
Wheat & oats	25	+	26	+	30	H.sat.
(Wheat 1931)	61	-	62	-	66	Fus.
Wheat & sweet clover & sweet clover	31	+	32	-	36	-
(Wheat & sweet clover 1931)	67	+	68	+	72	-

* + indicates successful isolations were made of the specified fungus.

\- indicates inability to isolate the specified fungus.

Discussion

In such an experiment as this it is impossible to prevent the soil becoming badly contaminated, thus creating a problem of how to determine whether or not the organisms still

persist. Three methods of isolation were used. Fusarium graminearum was isolated 19 times out of 26 attempts from the inoculated boxes against 5 times from the check. Helminthosporium sativum was isolated only 9 times out of 26 attempts against 3 times from the checks. Ophiobolus graminis was isolated 4 times out of 14 attempts as compared with 0 times in the checks. No successful isolations were made from the boxes inoculated with Leptosphaeria.

In general the results appear to indicate that Fusarium graminearum, Helminthosporium sativum and Ophiobolus graminis still persist in the inoculum after three years in the soil. It would require a large number of isolations made from each of the boxes to determine if the various rotations have had any effect on the persistence of these organisms in the soil. I would suggest that this experiment be allowed to run for five or six years before attempting to determine the relative effect of different rotations on the persistence of these fungi.

Experiments under controlled conditions were also conducted to determine the relative ability of cultures of known pathogenicity to persist in sterilized and unsterilized soil. Two experiments were conducted during 1930, and a similar one during 1931 in which the organisms were allowed to grow for two weeks before isolations were attempted, using the seedling method. The results of these experiments are summarized in Table 8.

TABLE 8.

Recovery of known foot-rotting pathogenes from sterilized and unsterilized soils two weeks after their introduction into these soils.

Organism introduced	Sterilized soil			Unsterilized soil		
	No.of seed-lings used	No.of seed-lings yield-ing fungi	Percent of seed-lings yielding fungi	No.of seed-lings used	No.of seed-lings yield-ing fungi	Percent of seed-lings yielding fungi
None - check	30	0	0	30	0	0
Helminthosporium sativum	51	37	72.5	125	9	7.2
Fusarium graminearum	50	40	80.0	125	8	6.4
Ophiobolus graminis	55	51	92.7	125	0	0
Leptosphaeria herpotrichoides	30	18	60.0	30	0	0

From the above table it may be seen that the four organisms tested were readily reisolated from the sterilized soil, but only Helminthosporium sativum and Fusarium graminearum were reisolated from the unsterilized soil. The positive evidence for the persistence of Helminthosporium sativum and Fusarium graminearum in unsterilized soil may be accounted for by the fact that these fungi are isolated with comparatively little difficulty. Negative evidence for Ophiobolus graminis and Leptosphaeria herpotrichoides is not regarded as conclusive, since these organisms, especially the former, are often difficult to isolate.

Persistence on seed and stubble stored in the laboratory

Several investigators have made observations on the ability of cereal foot-rotting fungi to overwinter under various conditions, but comparatively little other work has been done on their longevity. The object of these investigations has been to ascertain how long these fungi are capable of remaining alive in a dormant condition in seed and stubble, stored in a dry condition in the laboratory.

It is quite reasonable to believe that different organisms may vary considerably in their ability to survive in a dormant condition, and then proceed to grow when favourable conditions arrive. A condition analogous to this is found in the seeds of many of the flowering plants. Certain of these retain their viability for only a very short time, whereas others have been known to germinate after lying dormant for a period of over 40 years.

Christensen (3) found that Helminthosporium sativum remained viable in diseased seed for 6½ years. He was able to isolate the organism in the spring of 1921 from seed produced in 1914. However, he states that usually the hyphae in such seed are dead. He also made attempts to isolate the organism from badly diseased leaves, which had been stored in the laboratory for a similar length of time, but with negative results.

McKinney (17) made somewhat similar tests using barley seeds, and leaves from barley plants. He found that Helminthosporium sativum remained viable in infected seed which had been stored in the laboratory for 2 years, also that a small percentage of the spores taken from barley leaves stored in a herbarium for 3 years were still viable.

No work of this kind dealing with the longevity of Fusarium graminearum and Ophiobolus graminis has come to the attention of the writer. Maneval (15) worked on the longevity of various species of Fusarium and found that they remained alive for from five to eight years, when stored in a refrigerator at 10°C. However, Fusarium graminearum was not among the species tested.

In an experiment to determine if these fungi were able to live over long periods in seeds of wheat, samples from the 1918 and 1919 crops were tested with negative results. However, since these samples were not previously known to be infected, one is unable to say that the fungi were incapable of surviving such long periods. However, from experience it is found that most samples of grain are infected with such fungi as Alternaria, Helminthosporium, Fusarium spp., etc.

In another experiment an attempt was made to determine the length of time which foot-rotting fungi could remain viable in infested stubble, stored at room temperature in the laboratory. Fusarium, Helminthosporium

and Ophiobolus infested stubble which was collected from various points in the province during the 1928 plant disease survey was stored for 3½ years in the laboratory. Platings were made from the Fusarium and Helminthosporium infested stubble in October 1928 and again in January 1932. However, in the case of the Ophiobolus infested stubble no isolations were made in 1928, but samples showing typical "Take-all" symptoms were plated in January 1932. The results are given in Table 9.

TABLE 9.

Survival of foot-rotting fungi in naturally infested stubble stored in the laboratory.

Helminthosporium infested stubble			Fusarium infested stubble			Ophiobolus infested stubble	
Sample No.	Platings made Oct.1928	Jan.1932	Sample No.	Platings made Oct.1928	Jan.1932	Sample No.	Plating made Jan. 1932
130	+*	+	72	+	-	63	-
135	+	+	170	+	-	65	-
137	+	+	416	+	-	82	-
426	+	+	423	+	-	91	-
429	+	+	428	+	-	92	-

* Six isolations were made from each sample.
\+ Indicates successful isolations of specified fungus.
\- Indicates inability to isolate specified fungus.

The results obtained indicate that Helminthosporium sativum is capable of surviving at least 3½ years in dry

stubble stored in the laboratory. However, the Fusarium species and Ophiobolus graminis appear to be unable to survive for such a period when stored under dry conditions. In the case of Ophiobolus graminis one isolation was made from material which had been stored in the laboratory for a period of over one year.

These results are not directly comparable with field results, since the stubble was not exposed to the influence of weather conditions, such as moisture and temperature, which may be important factors influencing the longevity of these fungi under natural conditions.

The Overwintering of Ophiobolus graminis

The winter survival of Ophiobolus graminis depends almost entirely on its ability to survive either in the soil or on diseased plant remains, since it is not known to be carried over in the seed. In a study of this kind it is important to know what stage or stages of the fungus are capable of overwintering, in order that more effective control measures may be devised, e.g., if it is found that only the spores are capable of overwintering, factors affecting sporulation may well be considered.

The information we now have regarding this aspect of the cereal foot-rotting problem is not very extensive. Foster (7), working at this station during 1929-1930, found that the mycelium and spores of Helminthosporium sativum and

Fusarium graminearum, on diseased stubble, remained viable after exposure throughout the severe winter of 1929-'30 when temperatures as low as -49°F. were experienced.

In the experiment here reported attention has been confined to Ophiobolus graminis since practically no work of this nature has been done on this fungus under such severe conditions as exist in western Canada. Kirby (14) considers the fungus to overwinter in New York state in winter wheat seedlings infected by the germinating ascospores, shed from the perithecia at the time of planting. He gives no evidence regarding the ability of the fungus to overwinter in the soil as a saprophyte.

Davis (5) found that this organism was able to overwinter at Madison, Wisconsin, in both the mycelial and ascospore stages, but adds that there were cases where the fungus was killed. However, the winters in the above two states are usually much less severe than the average winter in Alberta. Fig. 3 shows the minimum monthly temperatures for the winter of 1931-32.

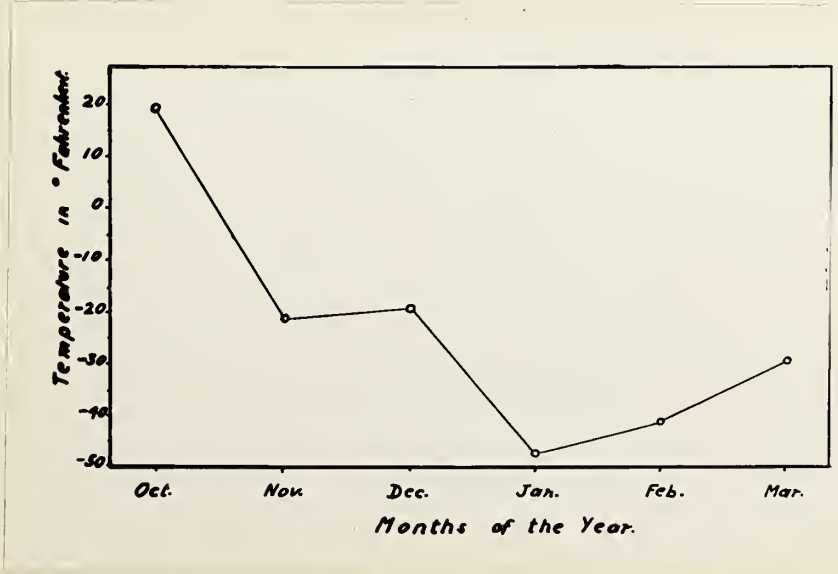

Fig. 3. Minimum monthly temperatures at Edmonton, Alta., 1931-32.

Ophiobolus graminis is only known to exist in two stages: the vegetative or mycelial stage, and the sexual or ascospore stage. Experiments to determine if these stages were capable of overwintering, both under natural and artificial conditions, were conducted during the winter of 1931-32.

Overwintering of the mycelium. Infected wheat stubble, from a naturally infested area on the University farm, was taken into the laboratory at intervals throughout the winter, and isolations made from it. The results are given in Table 10.

TABLE 10.

Results of attempts to isolate Ophiobolus graminis from infested stubble collected from the field at monthly intervals from Sept. 1931 to April 1932.

Date of collection	No. of pieces tested	No. of isolations
Sept. 20, 1931	12	2
Oct. 20, 1931	36	8
Nov. 28, 1931	36	0
Dec. 10, 1931	60	0
Jan. 20, 1932	36	1
Feb. 15, 1932	48	1
Mar. 28, 1932	110	4

The above results appear to indicate that the Ophiobolus mycelium was being killed as the winter progressed. However, the fact that some isolations were made after the extremely cold weather indicates that the mycelium is capable of surviving on infested stubble throughout our severe winters.

Further evidence of the ability of the mycelium of Ophiobolus to withstand low temperatures is furnished by the results of the following tests. Flasks of sterilized soil were inoculated with the fungus and placed outside on January 20, 1932. Isolations were made from these on February 20 and on March 24, 1932, and it was found that the fungus had survived in all cases.

Overwintering of the ascospores. For this study it was impossible to obtain naturally infected material on which spores were present, so pots of sterilized soil were inoculated with Ophiobolus ~~III~~ and seeded to Marquis wheat in the greenhouse during August 1931. Mature perithecia were developed on the base of the seedlings by the end of September, at which time the pots were placed outside on the surface of the soil. Germination tests of the spores, produced in the perithecia, were made in hanging drop cultures at intervals throughout the winter. Table 11 gives the germination percentages at the various dates.

TABLE 11.

Results of germination tests of Ophiobolus graminis spores exposed to the low temperatures throughout the winter 1931-'32.

Date of germination test	Percent germination in 48 hours at 20°C.
Nov. 30, 1931	25 - 30%
Jan. 10, 1932	30 - 35%
Feb. 10, 1932	25 - 30%
Mar. 24, 1932	3.3%

The above table shows a decided decrease in the percentage germination of the spores when tested on March 24, 1932, as compared with the test made on February 10, 1932. This is considered to be due possibly to the fact that most of the mature perithecia had shed their spores during the warm weather which occurred during the latter part of March, so that the spores obtained were immature, and possibly less able to withstand the low temperatures.

In order to test the resistance of spores produced in artificial culture, a petri dish containing a large number of perithecia produced at room temperature in the laboratory was placed outside in the open on January 15, 1932, while a similar one was kept inside at room temperature as a check. Germination tests were made on these spores on February 5 and 12, 1932. The results of these tests are shown in Table 12.

TABLE 12.

Effect of low temperatures on the germination of spores of Ophiobolus graminis produced in artificial culture at room temperature.

Date of germination test	Percent germination	
	Culture kept at room temperature	Culture kept outside
Jan. 10, 1932	45 - 50%	45 - 50%
Feb. 5, 1932	50 - 60%	0
Feb. 12, 1932	---	0.1%

The above results are based on counts of over 1000 spores germinating in hanging drops. It appears that the spores produced in culture are unable to withstand the low temperatures experienced during January.

A second experiment of a similar nature was conducted during February and March. Two sporulating cultures of Ophiobolus graminis of the same age, and grown under similar conditions were selected. One was placed directly outside on February 11, 1932. The second was kept in the laboratory at room temperature. Germination tests were made on spores from these cultures on March 24, 1932, the results of which are given in Table 13.

TABLE 13.

Effect of low temperatures on the germination of spores of Ophiobolus graminis produced in artificial culture at room temperature.

Date of germination test	Percent germination	
	Check - culture kept at room temperature	Culture placed outside
Feb. 11, 1932	50 -60	50 - 60
March 24, 1932	20 -30	5

From the results given in Tables 12 and 13, it appears that the spores produced in artificial culture are less resistant to low temperatures than are those produced on the plant.

FACTORS AFFECTING SAPROPHYTIC DEVELOPMENT

Effect of Crop Rotation on the Prevalence of Foot-rotting Fungi in the Soil

It has been found, both in practice and under experimental conditions, that soils which have been planted to wheat continuously often becomes unproductive. This condition has been considered by some people to be

due not to the lack of nutrients, but to the accumulation of certain micro-organisms which attack the plants causing a rotting of the roots, and basal parts of the stems. Probably one of the first to express such a view was Bolley (1), who in 1913 wrote a paper in which he attributed this unproductiveness to the accumulation of foot-rotting organisms, and referred to soil so infested as "wheat sick soil". However, Bolley made no attempt to demonstrate that such was the case, except that he found soil sterilization remedied the condition. This beneficial effect of soil sterilization has also been shown by Henry (10), who found that formaldehyde disinfection of soils gave increased growth of wheat seedlings over those on the untreated soil.

Greaney and Bailey (8), working at the Dominion Rust Research Laboratory, Winnipeg, made studies of the root-rots of cereals in Manitoba, and particularly on the influence of crop rotation on the fungous flora of wheat roots, and the pathogenicity of the fungi isolated from these roots. They made a comparison of the percentage of infected roots which were found to be present in samples taken at random from wheat plots from the various rotations. They identified only Fusarium and Helminthosporium species, the remainder being grouped as miscellaneous fungi.

From the results obtained they have drawn the conclusion: "That after consideration of the two years results, there does not seem to be any conspicuous

correlation between the severity of root-rot infection and the cultural practice involved; nor is there any convincing indication that there is any marked tendency for root-rotting organisms to accumulate in the soil during six years continuous cultivation of wheat".

This study on the prevalence of foot-rotting organisms in the soil has been undertaken in an attempt to ascertain whether or not certain of the major foot-rotting fungi vary in abundance in the soil of plots under different crops, and under different systems of rotation.

Before attempting to determine the numbers of these organisms in the various soils, some preliminary experiments were made in order to develop a suitable technique. Isolations were made from soils containing known pathogenes, and the effect of factors influencing infection were studied, e.g., the effect of temperature on the number of isolations. It was found that temperatures above 20°C. decreased the number of isolations, due apparently to the excessive growth of Rhizopus. This was partially overcome by growing the seedlings at a temperature of 15°-18°C. maintained by placing the seedlings in a bath through which cold tap water was allowed to flow.

It was thought that seedlings grown in the dark might yield more isolations than seedlings grown in the light, because of greater susceptibility to the

pathogenes, but this did not prove to be true. Hence the seedlings were grown in the light.

Influence of crop rotation. In a determination of the effect of crop rotation on the number of foot-rotting fungi in the soil, the following soils were selected for study:

1. Soil under virgin sod.
2. Soil from land cropped continuously to alfalfa (5 successive years).
3. Soil from a 1930 fallow which had been cropped to wheat in 1929.
4. Soil from land cropped continuously to wheat (4 successive years).

Procedure. Soil was collected under as sterile conditions as possible. It was placed in test-tubes and then sterile wheat seedlings were planted, one in each tube. In the first experiment approximately only 60 out of 100 seedlings grew. After two weeks growth, the seedlings were removed and attempts made to isolate pathogenes from them. A number of Helminthosporium sativum cultures were isolated together with certain other fungi, but it was decided to confine attention to this one pathogene because it can always be readily identified, whereas some of the other fungi, e.g., Fusarium species, are much more difficult to identify with certainty.

The above experiment was repeated twice using 100 tubes of each soil which gave very similar results. The results of these tests including only the pathogenic Helminthosporia are given separately, and in a summarized

form in Table 14.

TABLE 14.

Effect of crop rotation on the abundance of Helminthosporium sativum in the soil.

	Exp. No.	No. of attempts from each soil	Soils used			
			Virgin soil	Continuous alfalfa	Fallow following wheat	Continuous wheat
Helminthosporium sativum isolations obtained	1	60	0	0	2	3
	2	100	0	1	1	5
	3	100	0	0	4	6
	Summary	260	0	1	7	14

As a check on the sterility of the seedlings used in the first experiment, 40 were placed in test-tubes containing agar and grown for three weeks, during which time not a single contamination developed. As checks on succeeding batches of seedlings used, about 20 in each test were kept on agar in the dishes in which they were germinated, and it was found that if contamination had not shown up prior to germination, no contamination ever occurred.

From an examination of the summary in Table 14, it will be noted that there were considerably more successful isolations of Helminthosporium sativum from the

soil having grown four successive wheat crops than there were from any of the other soils. While it is admitted that 14 isolations out of 260 attempts is not a very high percentage, the constancy of the greater number from the continuous wheat, than from the other soils, is at least an indication that Helminthosporium sativum occurs more abundantly in the soils under continuous wheat culture.

With regard to the situation in the case of Ophiobolus graminis, an experiment was outlined to determine if this fungus was prevalent in soils which produced wheat heavily affected with "Take-all". Three soil samples were taken from a field at Stony Plain which showed 50-60 percent infection in localized areas, and a fourth sample was taken from a badly infested area on the University farm. Twelve seedlings were grown on each of the first three samples, and 50 on the fourth sample of soil. Attempts to isolate Ophiobolus from these seedlings gave negative results. However, the inability to isolate this fungus should not be considered as proof that it was not present in the soil, since field observations on the infection the following year, tend to show that more infection occurs in these areas provided conditions are favourable.

Effect of plant and soil extracts on the saprophytic growth of foot-rotting fungi

Many studies have been made on the effect of crop rotations on the development of cereal foot-rots, and the results obtained appear to indicate that infection is more severe in wheat following wheat than in wheat following certain other crops.

This is thought to be due to the larger amount of inoculum present in the soil when wheat is grown continuously, than when wheat follows a non-susceptible crop. The findings of Greaney and Bailey (8), however, do not appear to support this viewpoint, since they concluded that there does not seem to be any marked tendency for root-rotting organisms to acculumate in the soils during six years of continuous wheat cropping.

It was thought that the inoculum may be reduced after crops other than wheat, because other crop residues provide unfavourable substrata for the saprophytic growth of foot-rotting fungi. It has been reported* that the roots of certain plants excrete substances which are toxic to certain other plants and similarly they may contain and deposit in the soil substances deleterious to these fungi.

* Pickering, S.U. Ann. Bot. 31: 181 - 187. 1917

In order to test the latter hypothesis, wheat, barley, oats and rye were grown almost to maturity in pots of sterile soil, when extracts were made both from the tops and from the soils including the roots. The effect of these extracts on the growth of the foot-rotting fungi was then determined.

Effect of plant extracts. The extracts were prepared as follows: twelve grams of the tops of the above plants were extracted with 50 cc. of water in the autoclave for 30 minutes. The extracts obtained were used in the preparation of agar, made up according to Waksman's formula for soil extract agar. This was poured into petri dishes, 20 cc. per dish, and each was inoculated in the centre with a piece of mycelium of one of the following organisms: Helminthosporium sativum (Edmonton culture), Fusarium graminearum (New Norway culture), Ophiobolus graminis (Vermilion culture). These cultures were kept at room temperature, and measurements of the diameters of the colonies were made after four days in the case of the Fusarium, and after seven days in the case of Helminthosporium and Ophiobolus. The results are shown in Table 15, and also in Fig. 4.

TABLE 15.

Effect of plant extracts of different cereals on the saprophytic growth of foot-rotting fungi.

Extract from	Diameter of colonies in millimeters				
	1*	2*	3*	4*	Average
Fusarium graminearum after 4 days growth.					
Oats	72	71	72	73	72
Barley	78	82	79	80	80
Wheat	81	81	80	78	80
Rye	82	80	80	82	81
Helminthosporium sativum after 7 days growth.					
Oats	64	66	65	65	65
Barley	75	75	75	80	76
Wheat	78	75	74	77	76
Rye	84	83	83	82	83
Ophiobolus graminis after 7 days growth.					
Oats	24	29	30	29	28
Barley	--	65	68	45	59
Rye	58	69	60	60	62
Wheat	58	72	64	65	65

* replications.

From the above results, based on four replications, it is apparent that the oat plant extracts have a retarding effect on the growth of these foot-rotting fungi. This retarding effect was consistently exhibited by each of the fungi, and in all the replications used. This is possibly more or less what might be expected since oats are considered to be highly resistant to attack by most strains of *Ophiobolus graminis* and more resistant than the other

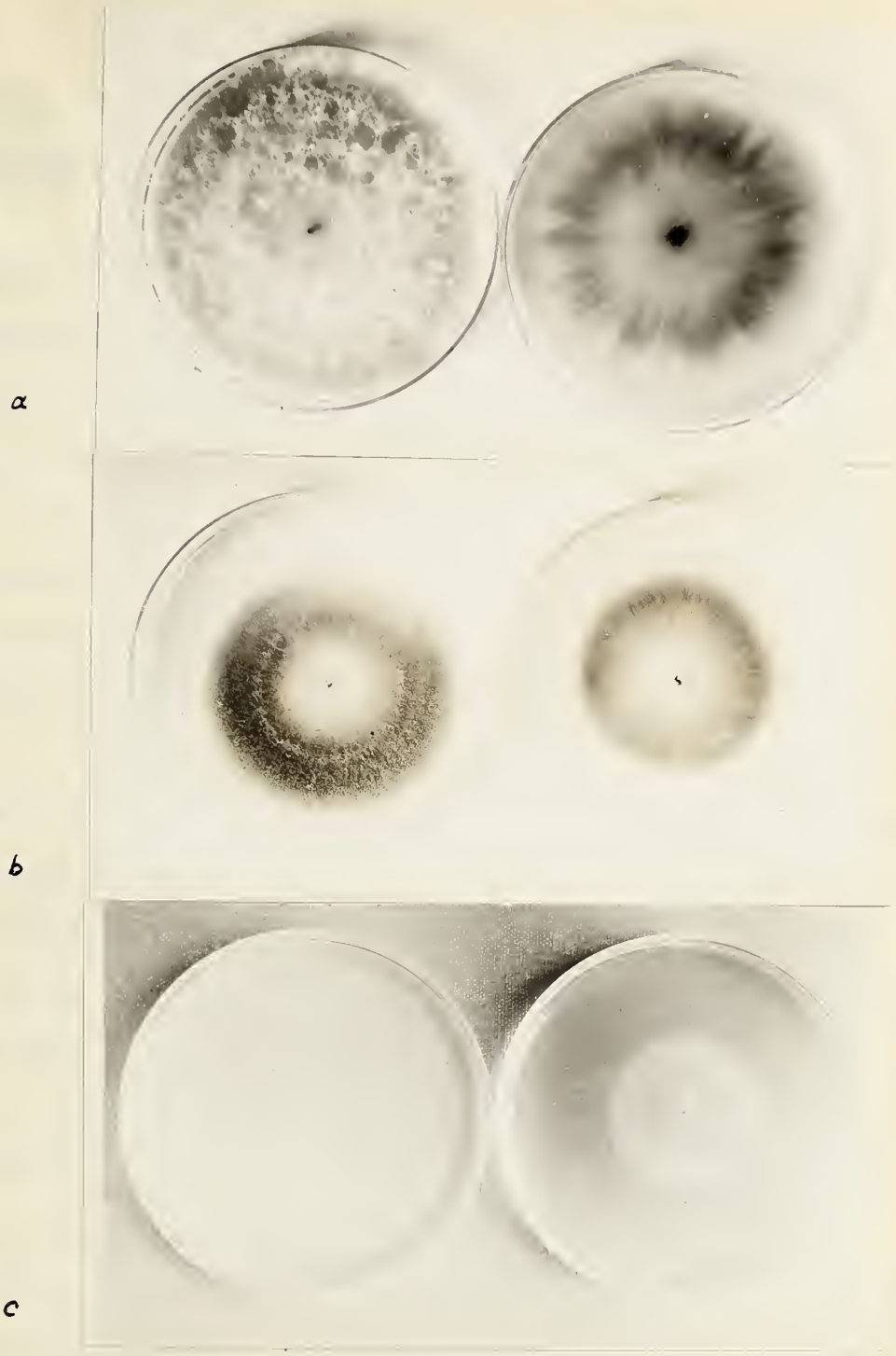

Fig. 4. Comparisons of the effect of wheat and oat plant extracts on the saprophytic growth of foot-rotting fungi.

a. Growth of Fusarium graminearum
b. Growth of Helminthosporium sativum
c. Growth of Ophiobolus graminis

Left. Wheat plant extract added to the medium.

Right. Oat plant extract added to the medium.

cereals used, to attacks by Helminthosporium sativum and Fusarium graminearum.

It remains to be proved that the substance or substances which cause this retarding effect on the saprophytic growth of these foot-rot fungi, is the same as that which gives resistance to the growing plants. If such is the case, it is very significant that they are not destroyed with the death of the plant. There is a possibility, however, that this lesser growth may be due simply to the less suitable nutrients provided by the oat extracts.

Effect of soil extracts. In this experiment an extract of the soil and roots of the above crops was prepared as follows: 1000 grams of soil and roots from each of the crops was extracted with 1000 cc. of water in the autoclave for 30 minutes. Agar was then made up, using 275 cc. of extract for 500 cc. of agar. Plates were poured and inoculated as in the previous experiment. The cultures were allowed to grow for eight days and then measured. It was found that the differences were negligible in the case of Fusarium and Helminthosporium, but in the case of the Ophiobolus the oat soil extract appeared to give the greatest growth. The results are given in Table 16.

TABLE 16.

Effect of soil extracts of different cereals on the saprophytic growth of Ophiobolus graminis.

Extract from soils having grown:	Diameter of colonies in millimeters after 11 days growth				
	1*	2*	3*	4*	Average
Oats	66	64	68	53	63
Wheat	49	58	58	60	56
Rye	58	61	55	42	54
Barley	45	57	52	60	53

* replications.

From the above table it would appear that the oat soil extract stimulated growth when compared with the soils from the other crops, but the variation between the replicates is as great as the variation between the crops, so the apparent differences probably are not very significant.

Effect of temperature on the saprophytic growth of different strains of Ophiobolus graminis and its possible relation to the infection of wheat seedlings

Previous studies on the temperature relations of Ophiobolus graminis have been made by Davis (5) who found that this organism would grow on agar at temperatures

ranging from 4° to 33°C. and that the optimum for vegetative growth ranged from 19° to 23°C. depending on the strain, as 3 strains from widely separated points were used in his study.

The results of experiments conducted during the past year, using strains collected in Alberta, tend to show that the various strains not only have different optimum temperatures, but also have different temperature ranges. The purpose of the experiments reported here was not to determine the optimum temperatures for growth and infection, but rather to determine if there were any marked differences in the saprophytic growth rates of the various strains, and if such occurred, to determine if these might be correlated with the severity of infection of wheat seedlings at corresponding soil temperatures.

The growth rates on potato dextrose agar were first determined for the various strains. Petri dishes, each containing 20 cc. of agar, were inoculated in the centre with mycelium from the four strains. A series of these plates was placed in incubators held at the following constant temperatures: 5°, 10°, 15°, 20°, 25°, 30° and 35°C., and allowed to grow for 10 days when measurements of the diameters of the colonies were made. A second experiment was made later using only the 15°, 20° and 25°C. temperatures. A summary of the results based on the two experiments is given in Table 17.

The following strains of Ophiobolus graminis were used:

Oph. III. This strain originated from a single ascospore obtained from a perithecium produced in artificial culture which was isolated from diseased wheat collected at Vermilion, Alta., in 1930.

Oph. IV. This strain came from a hyphal tip from a culture isolated from a perithecium found on diseased wheat collected at Athabasca, Alta., in 1930.

Oph. V. Isolated from mycelium in diseased wheat stems from material collected at Edmonton, Alta., in 1931.

Oph. VI. Isolated from mycelium in diseased wheat stems from material collected at Cardston in 1930.

TABLE 17.

Effect of air temperature on the growth of the mycelium of four strains of Ophiobolus graminis on potato-dextrose agar as shown by measurements of the diameters of the colonies.

Strain of fungus	Diameters of colonies grown at different temperatures						
	5°C.	10°C.	15°C.	20°C.	25°C.	30°C.	35°C.
Oph. III	5	27	58	64	81	0	0
Oph. IV	5	25	70	76	57	12	0
Oph. V	5	14	61	61	11	0	0
Oph. VI	5	19	63	74	83	0	0

The above figures are averages based on two experiments with 2 to 10 replications, the larger number of

replications were used at the 15°, 20° and 25°C. temperatures where the greatest amount of growth occurred.

From an examination of the results obtained it appears that the temperature range for the different strains of Ophiobolus graminis varies considerably, e.g., the maximum temperature for the growth of strain V appears to be close to 25°C., whereas for strain IV it appears to be over 30°C. Similarly the results suggest that the optimum temperatures for the growths of the four strains appear to vary more widely than has previously been thought.

A typical complete series of cultures grown at the various temperatures is shown in Fig. 5.

The influence of temperature on the infection of wheat seedlings by Ophiobolus graminis has been investigated by McKinney and Davis (18), who found that the optimum temperature for the development of the disease varied from 12°-16°C., the variation being effected by the soil moisture. This optimum they found to be lower than that for the growth of the host or the parasite, but they did not demonstrate any relationship between the saprophytic growth of the various strains used, and the severity of wheat seedling damage caused by these strains at different temperatures.

An experiment was conducted with the above points in mind. Inoculum of the four strains was grown for 14 days on a medium consisting of 50 gm. of sterilized

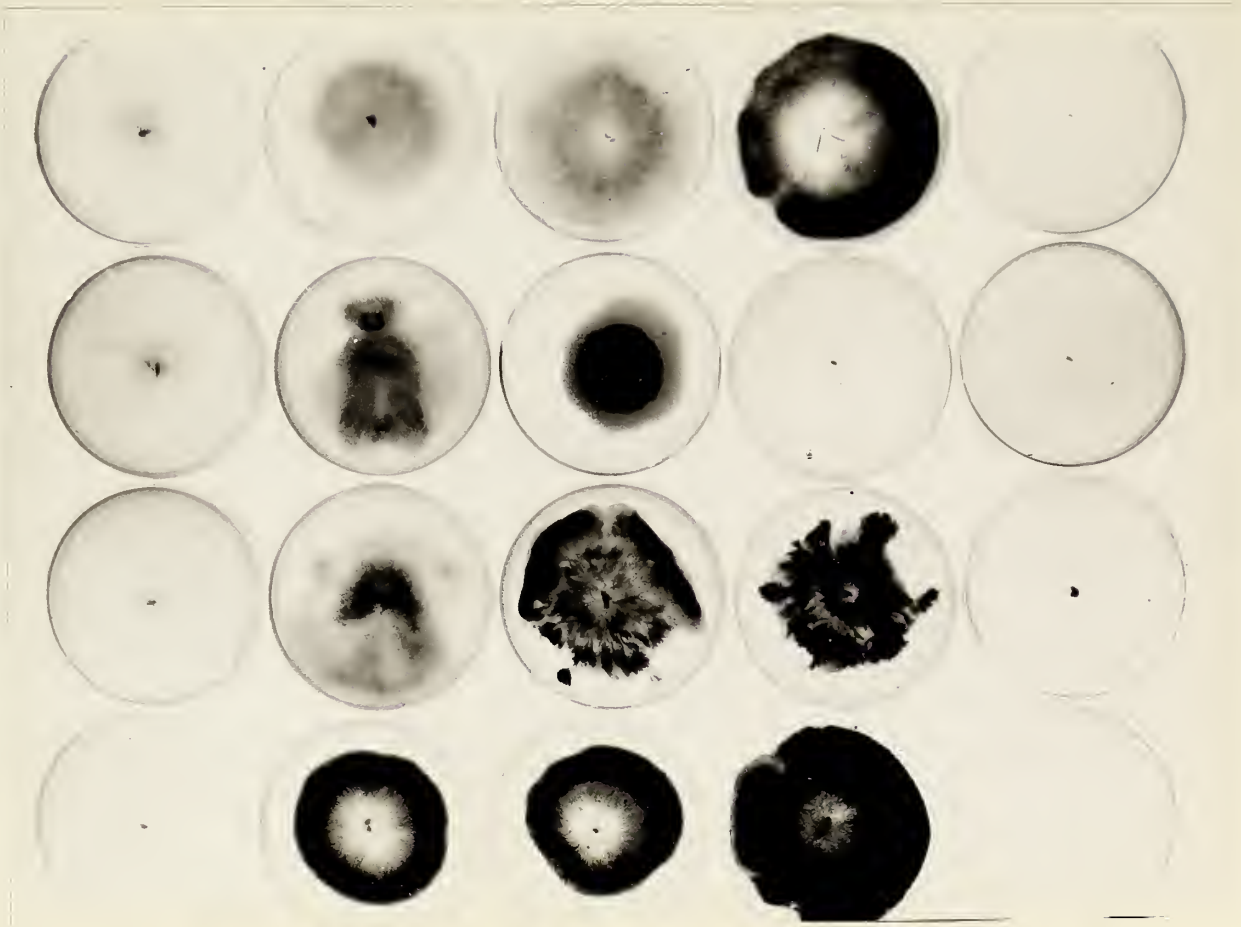

Fig. 5. Petri dish cultures of four strains of Ophiobolus graminis grown on potato-dextrose agar, and incubated at the following temperatures: left to right, 10°, 15°, 20°, 25° and 30°C. Row 1, Oph. VI; row 2, Oph. V; row 3, Oph. IV; row 4, Oph. III.

soil to which had been added 10 percent corn meal. This was added to six inch pots of unsterilized soil which were seeded to Marquis wheat, and then placed at the following temperatures 13°, 20°, 26°, and 32°C., in constant soil temperature tanks. Infection notes were made after 21 days. Numerical ratings as used by McKinney and Davis (18) were employed to obtain the degree of infection. The results are shown in Fig. 6 and are summarized in Table 18.

The above results show not only that there are considerable differences in the pathogenicity of the various strains of Ophiobolus graminis, but also if these results are compared with those given in Table 17, it will be noted that there is a direct relationship between the severity of the infection caused by each strain at different temperatures, and the vegetative growth on artificial media of that strain at corresponding temperatures.

With such a limited range of temperatures it is not possible to determine, with any degree of accuracy, the optimum temperature for infection with any particular strain. However, the results appear to indicate that the optimum temperature for infection varies directly with the optimum temperature for the growth of the pathogene

TABLE 18.

The effect of soil temperature on the severity of infection of Marquis wheat seedlings by four strains of Ophiobolus graminis.

Average soil temp. °C.	No. of plants	Oph. III Leaf blight rating	Oph. III Tiller base infection rating	Oph. III Root infection rating	Oph. III Average infection rating in %	No. of plants	Oph. IV Leaf blight rating	Oph. IV Tiller base infection rating	Oph. IV Root infection rating	Oph. IV Average infection rating in %
13°	40	0	39.1	41.7	33.3*	43	36.4	65.9	82.9	68.0
20°	40	13.3	40.0	54.2	41.3	35	72.4	95.2	95.2	91.1
26°	36	16.7	54.6	64.8	52.2	33	44.4	79.8	81.8	74.2
32°	34	4.9	19.6	41.2	26.4	29	1.1	26.4	32.2	24.3
		Oph. V				Oph. VI				
13°	40	0	15.8	39.1	23.1	42	31.0	54.0	57.9	51.5
20°	38	0	23.7	47.4	29.7	35	69.5	89.5	81.0	82.2
26°	35	2.9	21.0	39.0	23.7	38	50.9	93.9	71.1	76.1
32°	33	3.0	24.2	29.3	22.6	38	26.3	36.0	33.3	33.1

* The average infection rating is a weighted average of the three infection ratings measured.

Fig. 6. Effect of soil temperature on the development of seedling blight by different strains of Ophiobolus graminis.

Upper row. Oph. III at 32°, 26°, 20° & 13°C. Upper row. Oph. IV at 32°, 26°, 20° & 13°C.

Lower row. Oph. V at 32°, 26°, 20° & 13°C. Lower row. Oph. VI at 32°, 26°, 20° & 13°C.

as shown in Table 19, and Fig. 7, which are comparisons of the saprophytic growth rates of the four strains, and the severity of the infection caused by them.

TABLE 19.

The relation between saprophytic growth and pathogenicity of four strains of Ophiobolus graminis.

Strains	Comparative saprophytic growth rate	Comparative pathogenicity	Opt. temp. for saprophytic growth	Opt. temp. for infection of seedlings
Oph. V	1 ǂ	1 *	20°C.	20°C.
Oph. III	2	2	25°C.	26°C.
Oph. VI	3	3	25°C.	26°C.*
Oph. IV	4	4	20°C.	20°C.

* Applies only to tiller-base infection.

ǂ Indices computed from the entire temp. range

A relationship between the optimum temperature for disease development and the optimum temperature for the saprophytic growth of other pathogenes has been shown by various workers. However, that was not the main point it was hoped to demonstrate by this experiment, but rather, that there is a direct relationship between saprophytic growth and the severity of infection of seedlings by various strains at different temperatures. From an examination of the above table it would appear that such is the case,† but the evidence is insufficient

† This relationship is least evident in the case of Strain V which is only very slightly pathogenic even at the most favourable temperatures

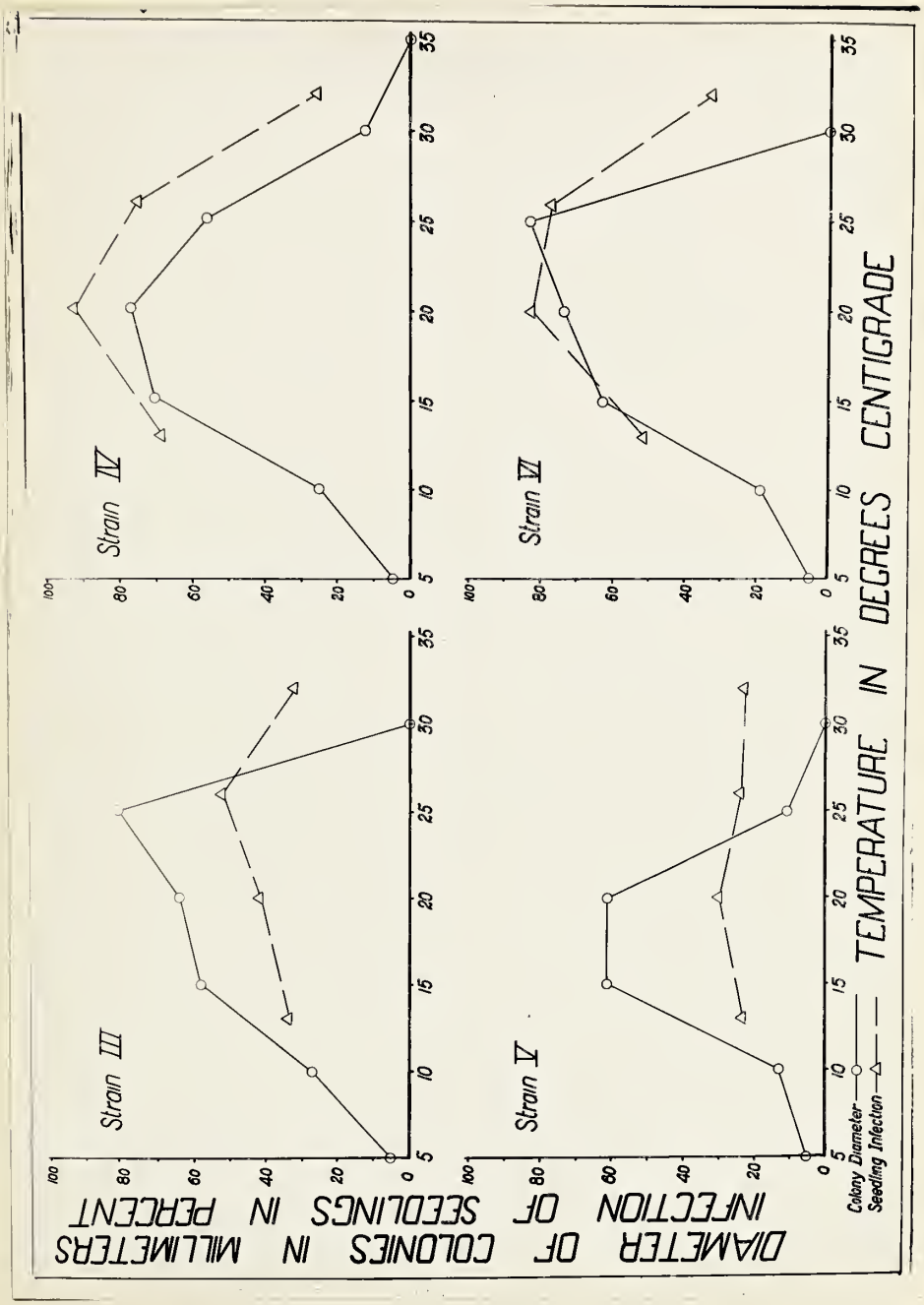

Fig. 7. Relation between the severity of wheat seedling infection caused by four strains of Ophiobolus graminis at different temperatures, and the vegetative growth on artificial media of those strains at corresponding temperatures.

to draw the conclusion that the one depends entirely on the other, because in all probability numerous other factors are involved.

Factors affecting sporulation in Ophiobolus graminis

The importance of the spores produced by Ophiobolus graminis has been previously stressed in the studies on the overwintering of this fungus. In this as well as in other connections it is of interest to know what factors affect sporulation, since this fungus normally does not sporulate readily.

Ophiobolus graminis is known to produce only ascospores, which are borne in flask shaped fruiting bodies known as perithecia. A mature perithecium contains numerous hyaline spore sacs or asci, each of which in turn contains eight hyaline threadlike, septate ascospores. (Fig. 8).

The perithecia when occurring on diseased plants are usually found embedded in the basal leaf sheaths. Kirby (13) states that perithecia are developed by the hundreds on the basal parts of wheat, barley, rye, and numerous grasses both wild and cultivated in the state of New York. They apparently occur much more abundantly there than in Alberta, as in certain years it is practically impossible to find a single perithecium on infected plants.

Fig. 8. Upper. Perithecium with asci and spores x 60.
Lower. Asci and spores x 160.

It naturally would be of interest to know precisely the factor or factors responsible for perithecial development in Ophiobolus. The following observations and experiments in this connection were therefore made.

It is thought that moisture conditions play an important part in perithecial development in Ophiobolus, because perithecia usually develop more abundantly in wet years or in wet districts than in dry ones. From our observations this appears to be true in Alberta. However, it has been observed that perithecia are not produced to the same extent on plants equally badly infected and growing under apparently similar conditions in the same field. Hence it is probable that other factors besides moisture affect development of this stage of the fungus.

Davis (5) found that certain strains of Ophiobolus graminis produced mature perithecia in culture whereas others did not. He suggested that this inability to sporulate was due to extreme lack of vigour, but considered that when other factors influencing sporulation are determined it may be possible to make these cultures sporulate. He found that by growing two colonies from the same single spore culture, on the same plate, he was able to get perithecial production where the two mycelia came together. These results suggest that nutritional factors or the products of metabolism may influence sporulation.

Heterothallism. The presence of heterothallic strains is known to occur in certain fungi including certain species of Mucor and Ascobolus. Ophiobolus

graminis has also been reported as heterothallic on the basis of Kirby's early work (14). He found that perithecia were never produced by monosporous cultures, but that when combinations of these cultures were grown together perithecia were often produced abundantly. However, more recent work by Davis (5) gives rather conclusive evidence that the fungus is homothallic, since he was able to produce perithecia from monosporous cultures. Recent work done at this laboratory confirms the work of Davis, since it was found that monosporous cultures produced perithecia abundantly under certain conditions.

It was considered that possibly the strains which Kirby used were heterothallic, but the work of Davis excludes this possibility, since he worked with monosporous cultures which he received from Kirby, and found them to produce perithecia. However, Kirby, in correspondence with certain other workers, has somewhat modified his published statements.

An experiment was outlined to determine if certain strains had a stimulatory effect on the sporulation of other strains when grown on artificial media. The following cultures were used: Oph. I and I^{I} which were single spore cultures, non-pathogenic and had never been known to produce perithecia either on artificial media or on infected plants in the greenhouse. The other two cultures used, Oph. III and IV, were hyphal tip cultures, very pathogenic, producing mature perithecia abundantly on infected plants, and producing large numbers of

immature perithecia on artificial media.

These strains were grown in all combinations, and it was found that there was no difference in the numbers of immature perithecia produced when two strains were grown in combination than when grown alone, and in no case were mature perithecia produced. When these cultures were last examined they had been growing for four months in the light and at room temperature.

It was found that perithecia develop abundantly on diseased seedlings grown in soil inoculated with either strain III or IV when grown in the greenhouse (Fig. 9).

Observations were made on the development of these perithecia produced on the seedlings which had been killed by the fungus. It was found that they commenced to develop on the base of the seedling in about three to four weeks and by the seventh week asci had started to form, but these did not mature until the ninth week, when there was an abundance of spores produced.

It has been pointed out in the case of certain fungi that other micro-organisms, e.g., bacteria and other fungi, sometimes influence sporulation. Considering especially the situation in which Ophiobolus perithecia are produced it seemed probable that other organisms might influence their development.

Fig. 9. Diseased seedlings bearing hundreds of mature perithecia.

In a review of the literature on this subject it was found that probably the first case to be reported was that of Molliard (19) who in 1903 reported variation in the number of perithecia produced by the fungus Ascobolus when it was grown in combination with a certain bacterium.

Sartory (22) in 1912 studied a very similar case, while working on a disease of the banana. He isolated a species of Aspergillus together with a bacterium which when cultured together on artificial media produced perithecia abundantly, but when the fungus was separated from the bacteria it remained sterile. Sartory explained his results on the supposition that the bacteria modified the culture media in some way, rendering it more favourable for sporulation.

It has also been found that perithecial production in certain fungi may be influenced by the presence of other fungi. McCormick (16) for example, found that perithecial production of Thielavia basicola was affected by Thielaviopsis basicola, a fungus usually associated with Thielavia basicola when found attacking tobacco plant roots. She found that when this organism was cultured alone very few perithecia were developed and only after several weeks, but when the organisms were cultured together perithecia were produced in abundance within ten days. She also tried the effect of a large number of other fungi and found that certain of them including species of Cladosporium and Aspergillus had a similar stimulatory

effect on Thielavia basicola.

Other observations along this line include those of Heald and Pool (9), and Wilson (29). These workers also found that when stimulation was obtained with the living fungi it was also obtained, although sometimes to a lesser extent, by sterile extracts of these fungi, provided the sterilization was not done by heat but through a bacteria proof filter.

During an earlier experiment, with Ophiobolus graminis, petri dishes inoculated with pure cultures were being grown, one of which became contaminated with a fungus, later identified as a species of Cladosporium, and also by an unknown bacterium which was designated B_1. On this plate perithecia were produced in abundance (Fig. 10) which upon examination were found to be mature, whereas on the uncontaminated plates no perithecia were visible.

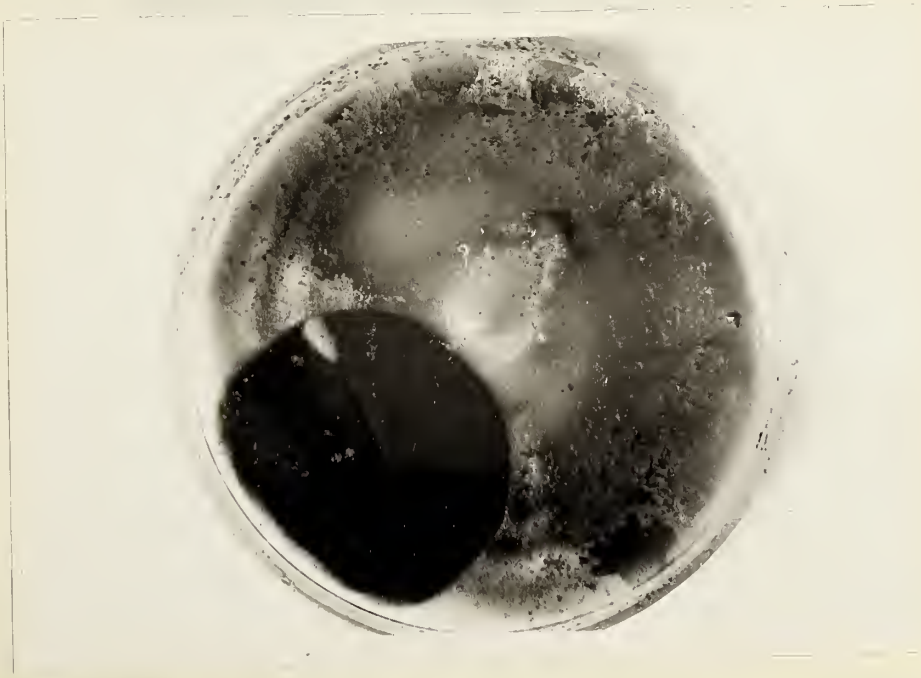

Fig. 10. Plate of Ophiobolus graminis contaminated with Cladosporium and an unknown bacterium (B_1) producing perithecia in abundance.

The bacterium and the fungus were isolated from the mixed culture, and combinations of Ophiobolus and the Cladosporium, also Ophiobolus and the bacterium were grown and notes made on perithecial production, Figs. 11 and 12.

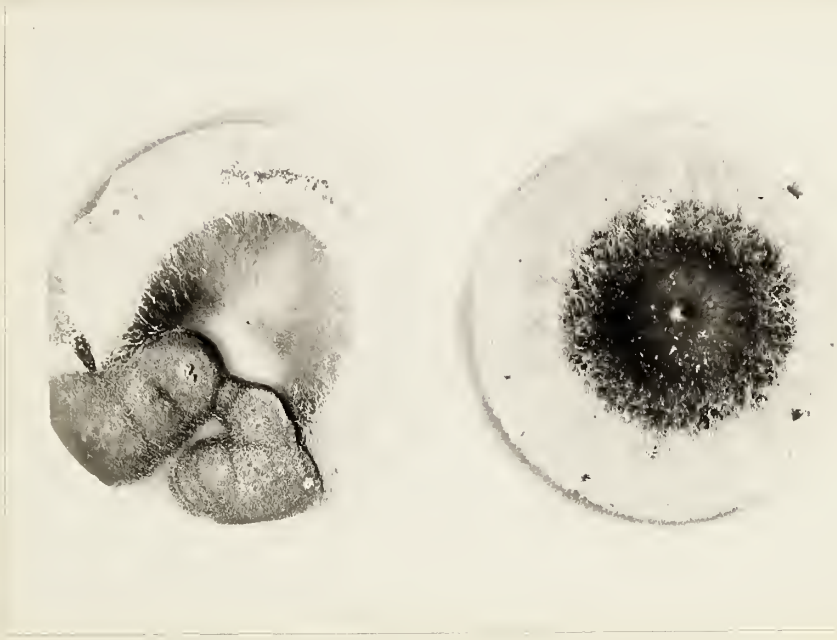

Fig. 11. Effect of Cladosporium on perithecial production.
a. Ophiobolus and Cladosporium.
b. Ophiobolus alone.

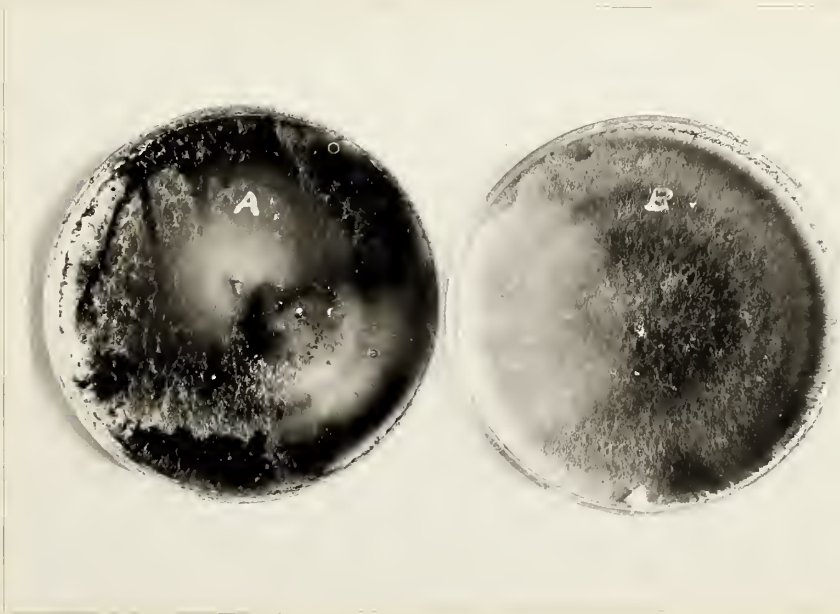

Fig. 12. Effect of bacteria on perithecial production.
a. Ophiobolus and the bacterium (B_1).
b. Ophiobolus alone.

From Fig. 11 it may be noted that the Cladosporium had no influence on perithecial production. In addition to Cladosporium the effect of the following other saprophytic fungi on perithecial development was tested: Penicillium, Alternaria and Aspergillus. Ten agar slants of Ophiobolus alone and in combination with each of the above were grown, but after three months' growth no stimulatory effect could be observed.

These organisms were also grown in combination with a culture of Wojnowicia graminis, which was known to produce pycnidia in culture, to determine if there was any effect on sporulation, but as in the case of Ophiobolus negative results only were obtained.

Water extracts of Cladosporium mycelia, which had been sterilized by passing through a Berkfeld filter, were added to cultures of Ophiobolus to determine if such extracts would have any effect on perithecial development, but as in the case of the living fungus negative results only were obtained.

From Fig. 12, it may be noted that the presence of the bacterium had a stimulatory effect. In the petri dishes containing the bacterium perithecia began to develop in about three weeks, whereas it was two weeks later before perithecia could be observed in the checks and only then in small numbers. Most of the perithecia in the checks did not mature whereas those in cultures contaminated with the bacterium matured in about four weeks.

Since it has been suggested that the products of metabolism of the associated organism are responsible for the perithecial stimulation, it was decided to try the effect of the addition of a nutrient solution in which a culture of the bacterium B_1 had been grown. Since others have found that heat is destructive to the substance capable of stimulating sporulation in other fungi, sterilization was effected by filtration. Three cc. of the filtered extract obtained was added to petri dishes containing approximately twenty cc. of potato dextrose agar, and these together with a series of checks were inoculated with Ophiobolus (Culture III), another similar series was also started using a single spore culture of Ophiobolus (Culture III, No. 1). These cultures were allowed to grow for five weeks at room temperature when notes regarding numbers of mature perithecia were taken. Out of the 24 cultures receiving the extract only one produced perithecia and no perithecia were observed in the checks. Only a few perithecia developed in this individual case indicating either that the extract did not possess this stimulatory property, or else only to a very small extent in comparison with the living bacteria.

Effect of temperature. Cultures of Ophiobolus alone and in combination with the bacterium B_1 were grown at different temperatures in order to ascertain the optimum temperature for perithecial development.

Four strains of Ophiobolus were used in this study; including Culture III, IV and two single spore

cultures obtained from Culture III.

Twelve petri dishes were inoculated with each of the above strains, and to six of these was added the bacterium B_1. Four series of these were made and placed at the following temperatures: 10°C., 15°C., 20°C. and 25°C. After four weeks the cultures were examined for the presence of perithecia, but none were found in any of the dishes. As the incubators were not lighted this may explain the failure of perithecial production, as it is known that light is an important factor in the production of certain kinds of spores.

These cultures were then placed at room temperature in the light, and allowed to grow for another four weeks. They were then examined for differences in perithecial numbers. Differences were observed in the cultures grown at the different temperatures, but the variations between the different temperatures were not as great as between the different cultures kept at the same temperature. However, one important observation was made, and that was that perithecia were observed to develop in all four strains containing the bacterium, whereas not a single perithecium could be found in the checks.

The fact that perithecia were produced by these monosporous cultures shows that strain III is homothallic.

Influence of ultra-violet light. It has been shown that ultra-violet light has a stimulatory influence on sporulation in a large number of fungi. Stevens (26, 27, 28) has done considerable work of this nature, studying

chiefly the organism Glomerella cingulata which causes the bitter rot of the apple.

The source of his ultra-violet light was a Cooper Hewitt quartz mercury arc, at 4.5 amps and 66 volts. He studied a single spore culture of Glomerella cingulata which had never been observed to produce perithecia, but on exposure to the ultra-violet light radiations for 1-5 seconds it produced perithecia literally by millions. He also studied the effect of ultra-violet irradiation on a large number of fungi, some of which responded readily, others not at all or only to a small extent; amongst the latter group Ophiobolus graminis was included.

Since it has been shown that different strains of Ophiobolus graminis vary in their reaction to different stimuli, it was decided to try the effect of ultra-violet radiations on two of the strains of this fungus used in the previous experiments. Strains III and IV were used. Petri dishes containing ten day old cultures of the above two strains were irradiated for varying periods of 2, 5, 10 and 15 seconds using a lamp similar to that used by Stevens*. The tops of the petri dishes were removed in order to allow the shorter light rays to strike the fungus.

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* The writer wishes to express his thanks to Dr. Hunter of the Biochemistry Department for making available the Cooper Hewitt quartz mercury arc lamp used in these experiments.

The response to the irradiation was not very great and only in one of the strains were perithecia produced to a small extent. It was found that mature perithecia developed in Culture III which had been irradiated for 10 and 15 seconds, but no perithecia developed in the checks or in those irradiated for the shorter periods.

INFLUENCE OF SAPROPHYTISM ON PATHOGENICITY

Pathogenicity of foot-rot fungi following periods of saprophytism

Since previous results indicate that foot-rotting fungi are present and apparently live considerable periods as saprophytes in the soil and in plant residues, it is important to know whether they retain their pathogenicity while living as saprophytes.

Pathogenicity of foot-rot fungi occurring naturally in the soil

In the isolations made from the various soils by the seedling method a large number of fungi were isolated most of which were saprophytes or possibly weak parasites. Most of these were not identified and were discarded,

but the Helminthosporium sativum cultures and those of Fusarium species were kept, and tests made of their pathogenicity in the following manner. Inoculum of the various cultures was grown for 14 days on a medium of 50 grams of sterilized soil in flasks. This was added to pots of sterilized soil, which were then seeded with Marquis wheat which had previously been treated with mercuric chloride (1-1000) for ten minutes, then washed in water. The pots were kept in the greenhouse at a temperature of 20 to 24°C., and the seedlings allowed to grow for two weeks when notes were made on the degree of infection. The results of this experiment are summarized in Table 20.

The above results show that all cultures of Helminthosporium sativum isolated from the soil were quite pathogenic. However, as was to be expected there was considerable variation in the pathogenicity of the various cultures ranging from 38 to 70 percent degree infection. With regard to the species of Fusarium tested, only a few cultures showed slight pathogenicity. These results are typical of those obtained in previous tests, but on which no actual figures are available.

It should be pointed out here that this method of isolation may only give the more pathogenic strains present, and if this is true it appears significant that no pathogenic cultures of Fusaria were isolated, indicating that Fusarium graminearum or other pathogenic species do not occur in the soil to the same extent as do pathogenic strains

TABLE 20.

Pathogenicity of cultures of Helminthosporium sativum and Fusarium species isolated from naturally infested soil.

Pathogenes	Culture No.	Seedling notes		
		Percent emergence	Height in cms.	Degrees of infection in percent
Check (no organism)		93.3	26.8	0
Helminthosporium sativum	Brooks culture (check)	65.0	12.0	66.8
" "	B 22*	71.6	12.5	62.1
" "	C 34	68.3	11.7	61.4
" "	C 37	51.6	8.5	70.0
" "	C 42	56.7	9.7	67.1
" "	C 43	56.7	11.4	59.6
" "	C 44	58.3	10.5	61.4
" "	C 45	53.3	9.4	63.6
" "	C 46	81.7	15.4	38.6
Fusarium graminearum	New Norway culture (check)	80.0	16.2	44.8
" spp.	B 24	88.9	21.9	5.7
" "	B 25	93.3	23.1	0
" "	C 33	98.0	21.4	0
" "	C 36	91.1	22.3	0
" "	C 43	93.3	21.3	2.4
" "	A 4	98.0	22.5	0
" "	A 5	88.9	21.9	0
" "	A 13	95.6	21.9	3.3
" "	A 12	91.1	21.1	6.7

* The cultures tested in this experiment were obtained from a soil sample taken from a wheat plot on the University farm.

of Helminthosporium sativum. Also as previously stated this method of isolation did not yield any cultures of Ophiobolus graminis, indicating the possible absence of this organism from the soils tested.

Effect on pathogenicity of culturing known strains of Helminthosporium sativum and Fusarium graminearum in the soil. In this experiment it was decided to determine if the saprophytic growth in unsterile soil, and the association with the saprophytic micro-organisms in the soil had any effect on the pathogenicity of known cultures of Helminthosporium sativum and Fusarium graminearum.

Flasks containing 50 gm. of unsterile soil were inoculated with Helminthosporium sativum and Fusarium graminearum of known pathogenicity. The cultures were allowed to develop for two weeks, when sterile seedlings were planted in the flasks, and these in turn allowed to grow for two weeks, when isolations were made from them. Five cultures of Helminthosporium sativum and six of Fusarium graminearum were recovered. Pathogenicity tests, as previously described, were then made on these and on the original cultures and the results compared. Table 21 gives a summary of the results obtained.

TABLE 21.

Effect of saprophytic growth in unsterile soil on the pathogenicity of known cultures of Helminthosporium sativum and Fusarium graminearum.

Pathogenes	Seedling notes		
	Percent emergence	Height in cm.	Degrees of infection in percent
Check - no organism	86.5	22.8	0
Helminthosporium sativum No. 7 (Original culture)	44.5	7.5	76.0
Helminthosporium sativum No. 16 (reisolated from unsterilized soil)	56.0	8.3	68.6
Helminthosporium sativum No. 13 (reisolated from unsterilized soil)	45.0	5.8	78.3
Helminthosporium sativum No. 14 (reisolated from unsterilized soil)	46.0	6.3	61.8
Helminthopsorium sativum No. 11 (reisolated from unsterilized soil)	60.0	7.1	67.4
Fusarium graminearum No. 2 (original culture)	5.0	1.0	98.8
Fusarium graminearum No. 8 (reisolated from unsterilized soil)	12.0	1.0	97.2
Fusarium graminearum No. 11 (reisolated from unsterilized soil)	13.0	1.0	96.5
Fusarium graminearum No. 7 (reisolated from unsterilized soil)	14.0	1.0	97.0
Fusarium graminearum No. 15 (reisolated from unsterilized soil)	13.5	1.0	95.6
Fusarium graminearum No. 16 (reisolated from unsterilized soil)	12.0	1.0	96.5

The above results show that the saprophytic growth, and the association with other saprophytic organisms in unsterile soil had no appreciable effect on the pathogenicity of known cultures of Helminthosporium sativum and Fusarium graminearum. These results are important in the light of Henry's work (11), who found that the presence of other micro-organisms in the unsterilized soil reduced the severity of infection of wheat seedlings by Helminthosporium sativum and Fusarium graminearum. In the light of the above results it would appear that this reduction in severity of infection is probably not attributable to a change in pathogenicity of the pathogenes.

SUMMARY

1. Improved methods of isolation of foot-rotting fungi from the soil and from diseased plants were developed.

2. The persistence of Helminthosporium sativum and Fusarium graminearum for 2½ years in inoculated soil, under field conditions was demonstrated.

3. Helminthosporium sativum, Fusarium graminearum, Leptosphaeria herpotrichoides and Ophiobolus graminis were readily reisolated from sterilized soil, whereas only the former two were reisolated from unsterilized soil.

4. Differences were found in the ability of Helminthosporium sativum and Fusarium graminearum to persist on stubble stored for 3½ years in the laboratory. Helminthosporium sativum proved to be the more persistent.

5. Ophiobolus graminis was found to overwinter to a certain extent in both the mycelial and ascospore stages.

6. Evidence was obtained which indicates that the pathogene Helminthosporium sativum occurs more abundantly in soils cropped continuously to wheat than it does in soils under certain rotations.

7. The saprophytic growth of foot-rotting fungi on oat plant-extract agar was found to be slower than on wheat plant-extract agar. This may be due to an inhibitory effect of the oat extract, or simply to the less suitable nutrients provided by the oat extract.

8. Various strains of Ophiobolus graminis appear to differ in their reaction to temperature, both as regards saprophytic growth on artificial media, and as regards the infection of wheat seedlings.

9. The strains of Ophiobolus graminis studied showed considerable variation both in saprophytic growth rates and in pathogenicity, and those capable of most rapid saprophytic growth proved to be the most virulent.

10. A positive correlation was found between the severity of disease caused by each strain of Ophiobolus at different temperatures, and the rate of vegetative growth on artificial media of that strain at corresponding temperatures.

11. Factors affecting sporulation in Ophiobolus graminis were studied. It was found that the presence of an unidentified bacterium stimulated the abundant production of perithecia in artificial culture. The influence of

ultra-violet light on sporulation was also studied. Exposure for 2, 5, 10 and 15 seconds did not stimulate perithecial production to any great extent.

12. All cultures of Helminthosporium sativum isolated from the soil proved to be quite pathogenic, whereas the Fusarium species obtained from the soil were only slightly pathogenic or non-pathogenic.

13. The association of known cultures of Helminthosporium sativum and Fusarium graminearum with the saprophytic organisms of the soil had no appreciable effect on their pathogenicity.

ACKNOWLEDGMENT

In conclusion the author wishes to express his thanks to his advisor, Dr. A. W. Henry, for directing the investigation, and for helpful suggestions in the preparation of this manuscript; also to the National Research Council for financial assistance received.

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